FIG.2

Step B

$$P^{6} \stackrel{P^{6'}}{\downarrow}$$
 $P^{5}O$
 $P^{3}O$
 $P^{2}O$
 $P^{2}O$
 $P^{2}O$
 $P^{2}O$
 $P^{3}O$
 $P^{2}O$
 $P^{3}O$
 $P^{2}O$
 $P^{3}O$
 $P^{2}O$
 $P^{3}O$
 $P^{3}O$
 $P^{3}O$
 $P^{3}O$
 $P^{4}O$
 $P^{5}O$
 P^{5

trifluoromethanesulfonate

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FIG.3

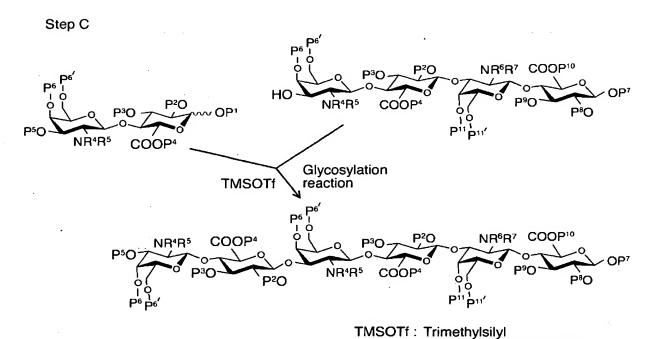
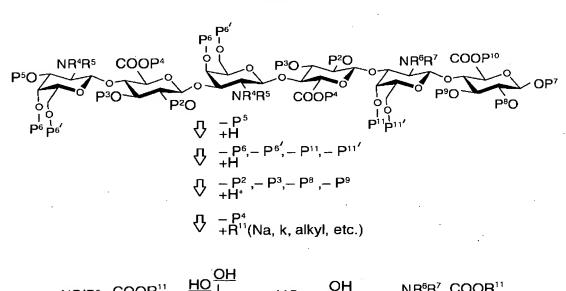
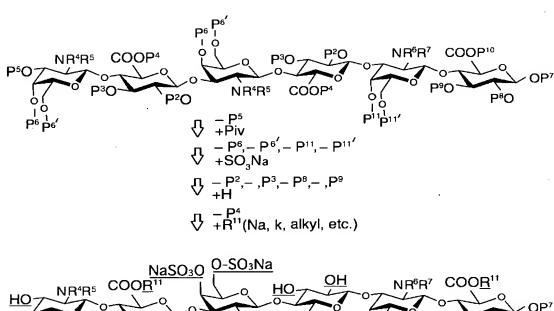
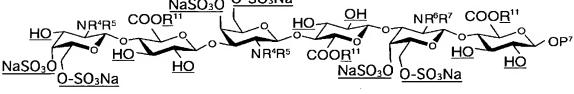


FIG.4









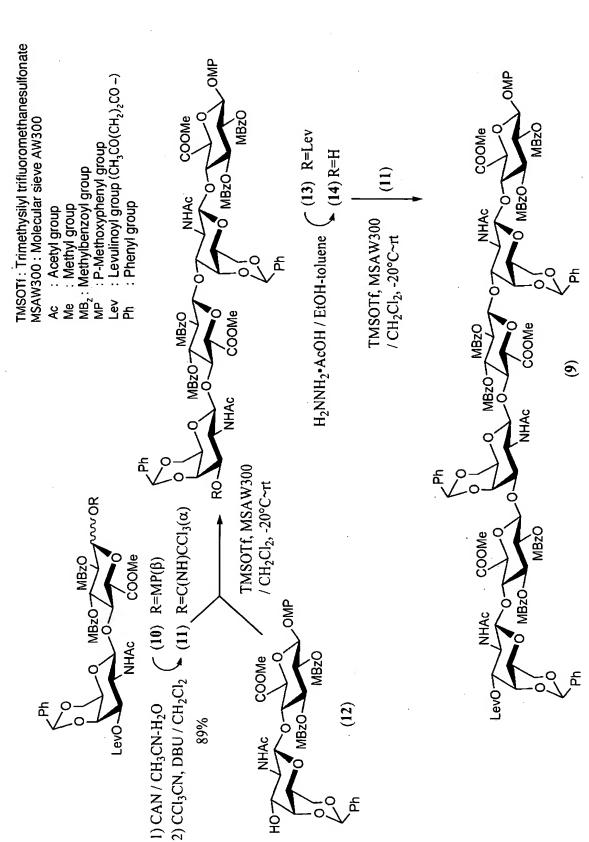
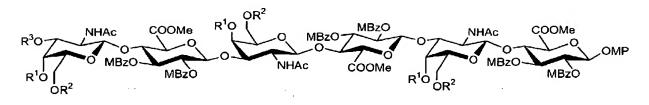


FIG.6-2



(9)
$$R^{1}$$
, R^{2} =>CHPh, R^{3} = Lev
(15) R^{1} , R^{2} ->CHPh, R^{3} - H
(16) R^{1} , R^{2} =>CHPh, R^{3} = Piv
(17) R^{1} = R^{2} = R^{3} = H
(18) R^{1} = R^{2} = 11, R^{3} = Piv
(19) R^{1} = R^{2} = SO₃Na, R^{3} =Piv

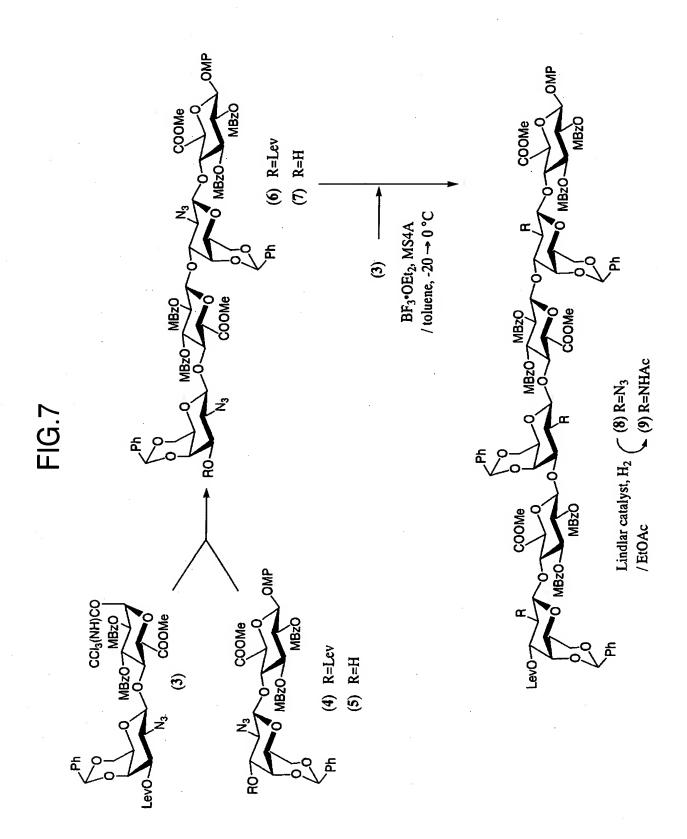
 Acetyl group
 Methyl group
 Methylbenzoyl group Ме

MBz MP

: P-Methoxyphenyl group : Levulinoyl group (CH₃CO(CH₂)₂CO –) : Pivaloyl group ((CH₃)₃(CO –) : Phenyl group Lev

Piv

Abbreviations: Me, CH₃-; MBz, p-MeC₆H₄CO-; MP, p-MeOC₆H₄-; Ac, CH₃CO-; Ph, C₆H₅-.



<Test Method>

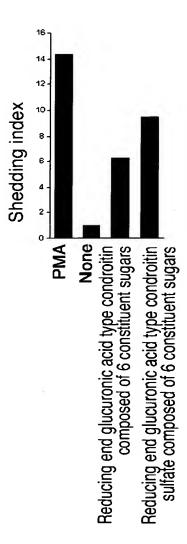
- ↓ MIA PaCa-2 (human pancreatic carcinoma cell line) is plated on 24-well plate (1 x 10⁵ / well)
- Incubation (37°C, overnight)
- \downarrow Incubation (37°C, 30min) with 10 $\mu{\rm M}$ cysteine protease inhibitor (MQ132) added
- ↓ Incubation (37°C, 60min) wth RCE6 or RC6 added
- positive control : Incubation (37°C, 30min) with Phorbol myristic acid ester added
 - · negative control: Nothing is added
- Cells are collected after lysated with SDS buffer
- ↓ SDS PAGE
- ↓ Western blotting assay

Primary antibody: anti-human cytoplasm CD44 rabbit polyclonal antibody Secondary antibody: horseradish peroxidase conjugated goat anti-rabbit IgG

Reducing end glucuronic acid type condroitin sulfate composed of 6 constituent sugars Reducing end glucuronic acid type condroitin composed of 6 constituent sugars None

25-kDA CD44Cleavage Product

FIG.10



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